Method Development for Chiral LC/MS/MS Analysis of Acidic Stereoisomeric Pharmaceutical Compounds with Polysaccharide-based Stationary Phases

Liming Peng, Swapna Jayapalan, and Tivadar Farkas

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

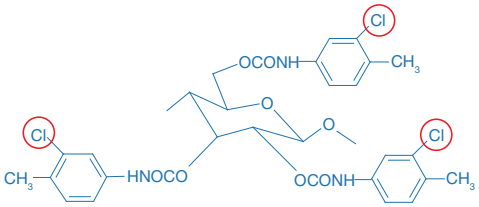
PO87820611_L_1

Introduction

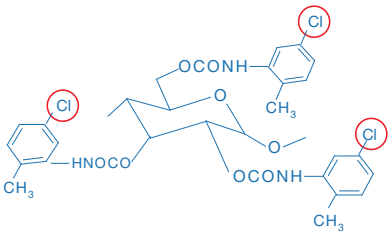
Developing simple and straightforward reversed phase (RP) chiral LC separations coupled with highly sensitive MS detection is required for conducting drug metabolism and pharmacokinetic studies of stereoisomers and a challenging part of the drug discovery process. We have presented our successful results on using polysaccharide-based stationary phases coupled with API-MS/MS detection for the analysis of various basic and neutral pharmaceutical compounds in reversed phase elution using NH_4HCO_3 or NH_4Ac as buffer salts and acetonitrile or methanol as organic modifiers.[1] This investigation is extended to the effectiveness of using an acidic mobile phase for the separation and detection of acidic stereoisomers by ESI or APCI LC/MS/MS and to method development of Chiral LC/MS/MS analysis using polysaccharide-based chiral stationary phase (CSPs).

Figure 1. Structures of Chiral Phases

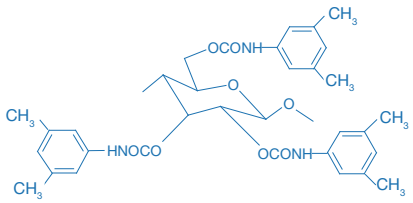
Lux® Cellulose-2 or-4
Cellulose tris (3-chloro-4-methylphenylcarbamate) or
(4-chloro-3-methylphenylcarbamate)



Lux® Amylose-2
Amylose tris (5-chloro-2-methylphenylcarbamate)



Lux® Cellulose-1
Cellulose tris (3,5-dimethylphenylcarbamate)



Lux® Cellulose-3
Cellulose tris (4-methylbenzoate)

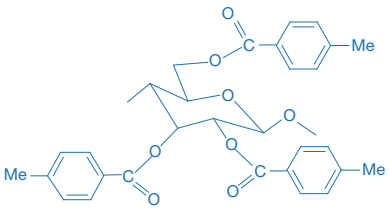


Figure 2. Molecular Structures of Acidic Racemates

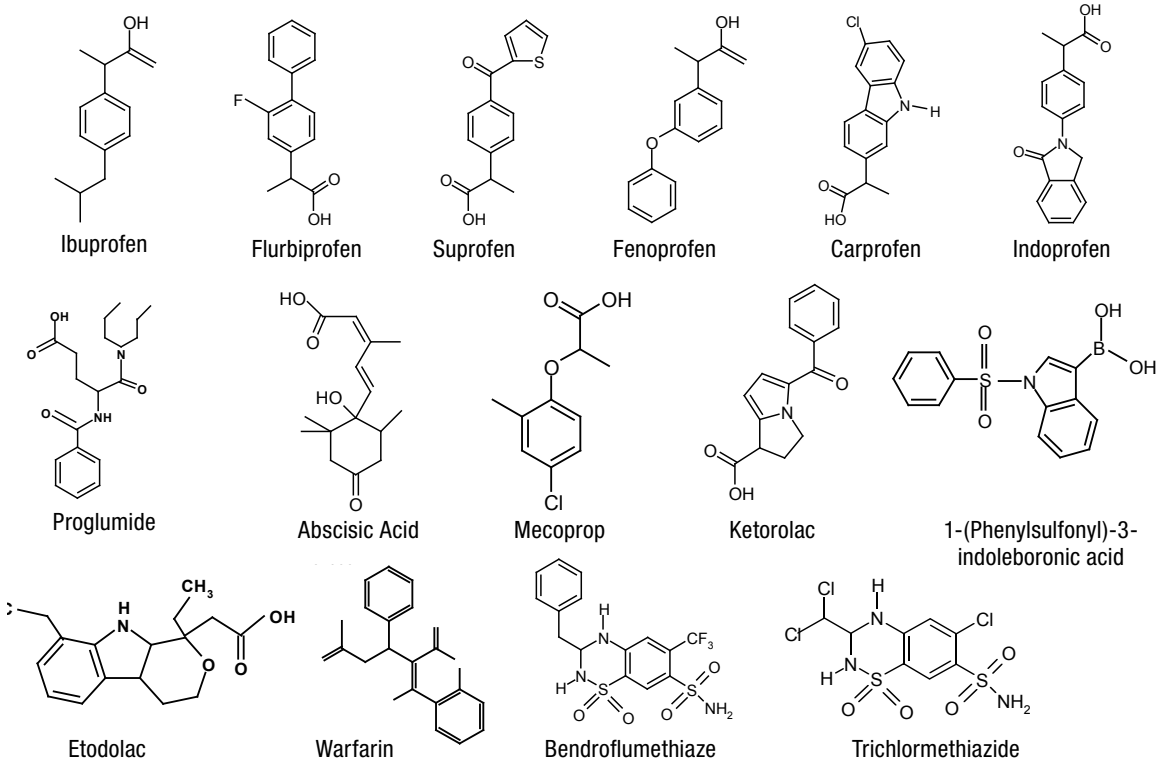


Table 1. MRM Transitions and Concentrations of Acidic Racemates

Compound	IS and MRM	Conc.*	Compounds	IS and MRM	Conc.*
Ibuprofen	ESI^-205.1/160.1	100	Abscisic acid	$APCI^-$263.0/152.7	50
Flurbiprofen	ESI^-243.0/198.7	50	Mecoprop	ESI^-214.1/141.7	50
Suprofen	ESI^+261.1/111.0	50	Ketorolac	ESI^-254.0/209.8 ESI^+256.2/105.0	50
Fenoprofen	$APCI^-$241.0/196.8	100	Etodolac	ESI^-286.1/242.0	50
Carprofen	$APCI^-$272.8/228.8	100	Warfarin	ESI^-307.1/160.9 ESI^+309.2/163.1	50
Indoprofen	ESI^+282.1/236.1	50	Bendroflumethiaze	ESI^-420.1/77.9	50
Proglumide	ESI^-333.1/120.9	50	Trichlormethiazide	ESI^-377.8/241.6	50
1-(Phenylsulfonyl)-3-indoleboronic acid	ESI^-300.0/209.8	100	* Conc. (ng/mL)		

Experimental Conditions

Instrumentation

HPLC System: Agilent® 1200 series

Pump: G1312B (Binary Pump)

Autosampler: G1337C HP-ALS-SL

MS Detector: AB SCIEX™ API 4000™ LC/MS/MS -Turbo V™ Source with ESI or APCI probe

MS Detection

TurbolonSpray® —ESI or APCI in Positive or Negative Ion Mode; MRM transition

HPLC Conditions

Flow Rate: 0.2 mL/min (3 µm, 150 x 2.0 mm) or 1.0 mL/min (5 µm, 250 x 4.6 mm), flow split to 0.25 mL/min into MS/MS

Injection Volume: 5 µL (150 x 2.0 mm) or 20 µL (250 x 4.6 mm)

Columns:

Lux® Cellulose-1 3 µm	150 x 2.0 mm
Lux® Cellulose-2 3 µm	150 x 2.0 mm
Lux® Amylose-2 3 µm	150 x 2.0 mm
Lux® Cellulose-4 5 µm	250 x 4.6 mm
Lux® Cellulose-3 5 µm	250 x 4.6 mm
Kinetex® 2.6 µm C18	50 x 2.1 mm (used for achiral analysis)

Mobile Phases:

1. Acetonitrile/Methanol: 0.1 % Formic acid (FA)
2. Acetonitrile/Methanol: 5 mM NH_4HCO_3 – achiral analysis
3. Acetonitrile/Methanol: 5 mM $HCOONH_4$ (NH_4FA) – achiral analysis
4. Acetonitrile/Methanol: 5 mM CH_3COONH_4(NH_4AC)- achiral analysis

Figure 3. LC/MS/MS Responses of Acidic Racemates in ESI$^-$ with Different Additives and Methanol

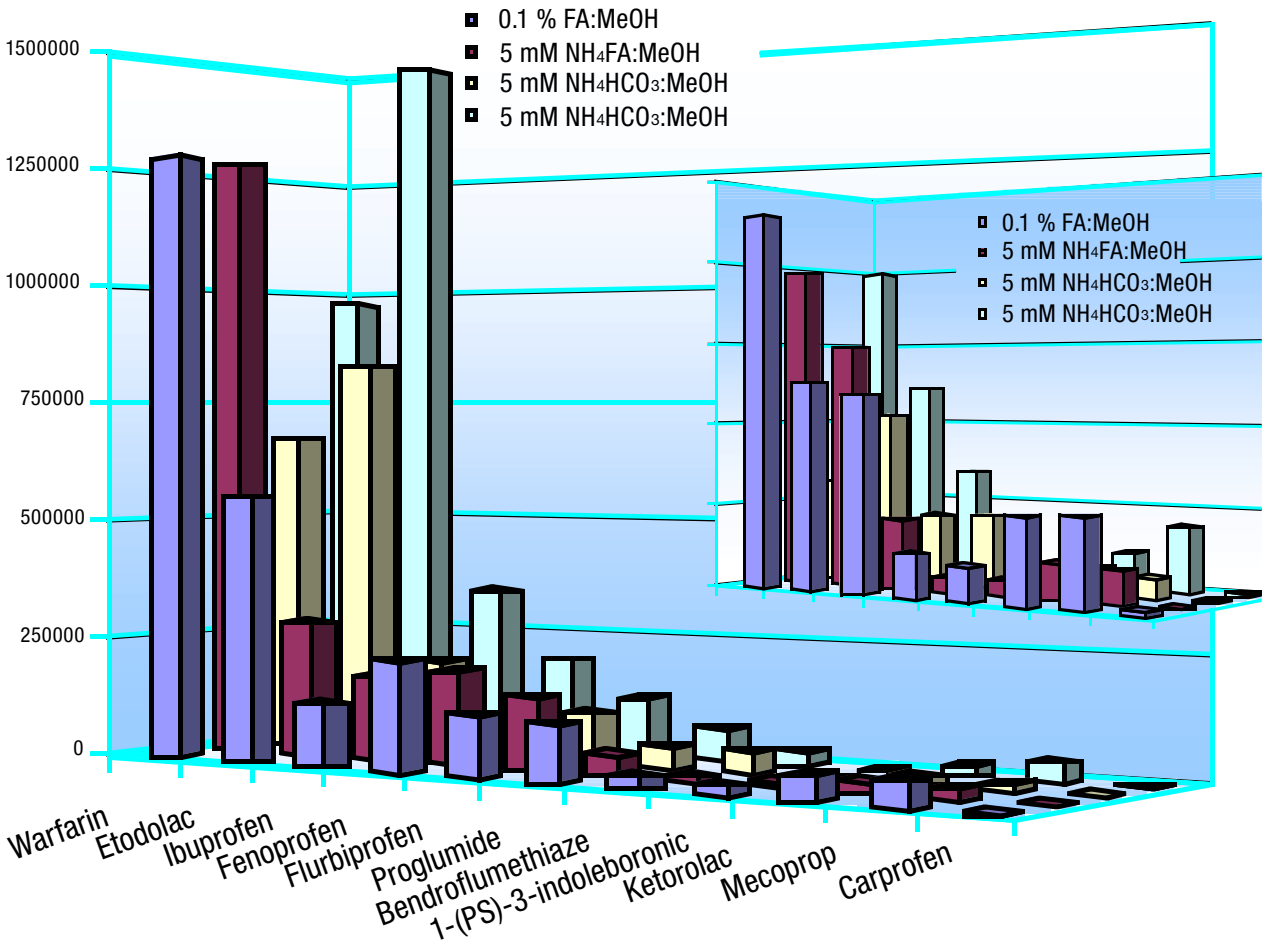


Figure 4. **LC/MS/MS Responses of Acidic Racemates in ESI- with Different Additives and Acetonitrile**

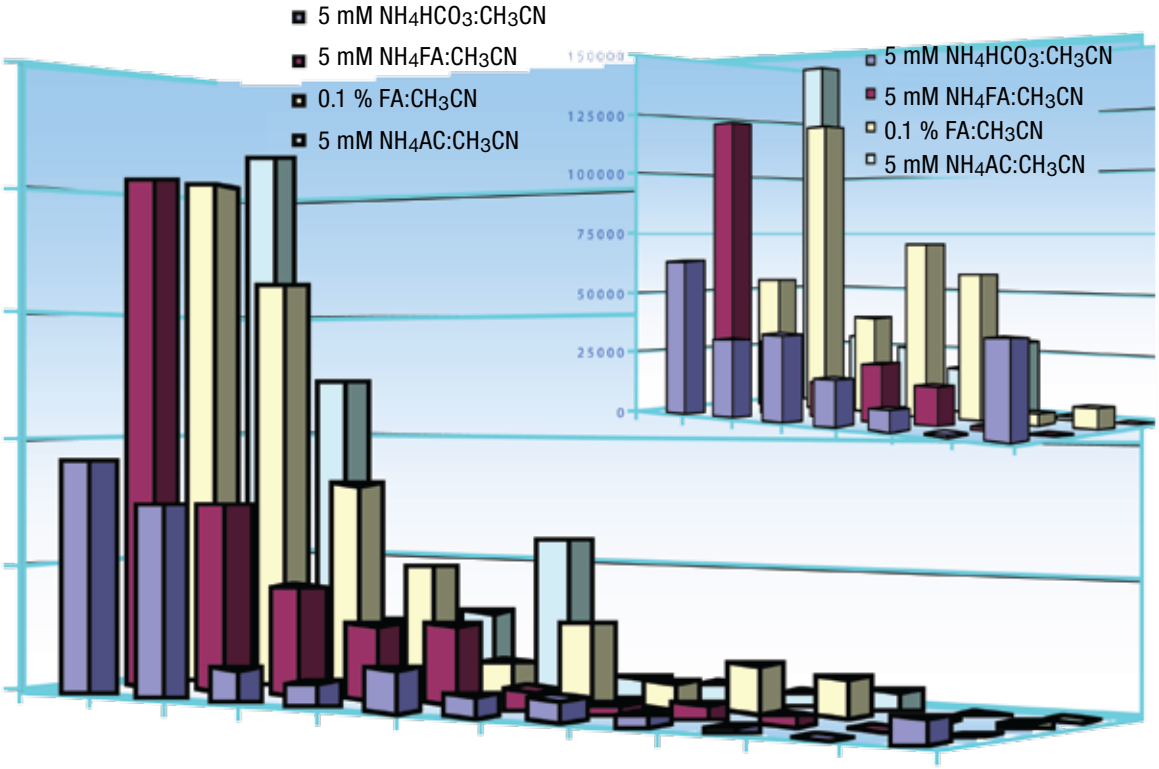
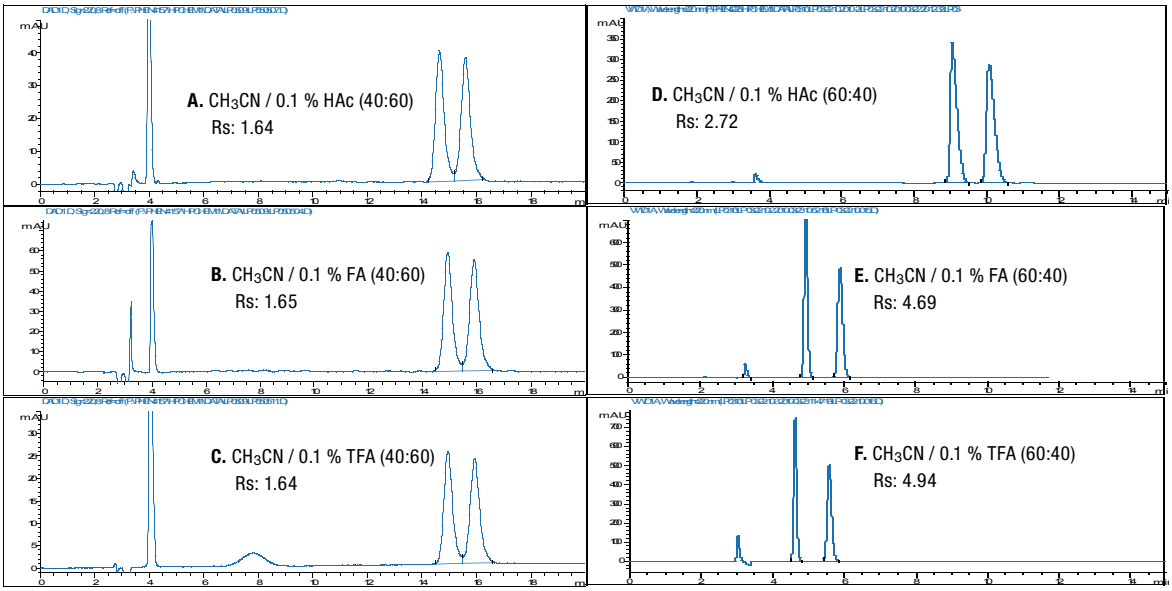


Figure 5. The Effect of Acidic Additives on the Enantioseparation of Acidic Racemates in RP

Bendroflumethiaze
Lux® 5 µm Cellulose-1 250 x 4.6 mm

Flurbiprofen (pK_a 4.33)
Lux® 5 µm Amylose-2 250 x 4.6 mm

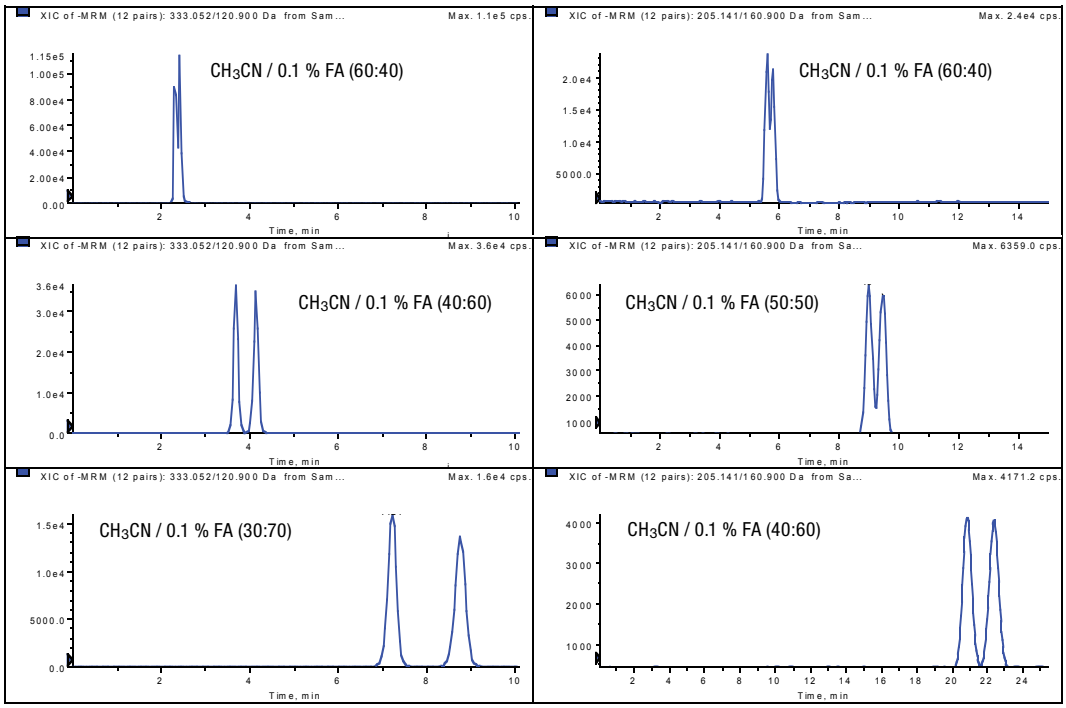


Conc.: 250 µg/mL; Flow Rate: 1.0 mL/min; Injection: 10 µL; Detection: UV @ 220 nm
HAc - Acetic Acid TFA - Trifluoroacetic Acid

Figure 6. The Effect of Organic Modifier on the Enantioseparation of Acidic Racemates in RP

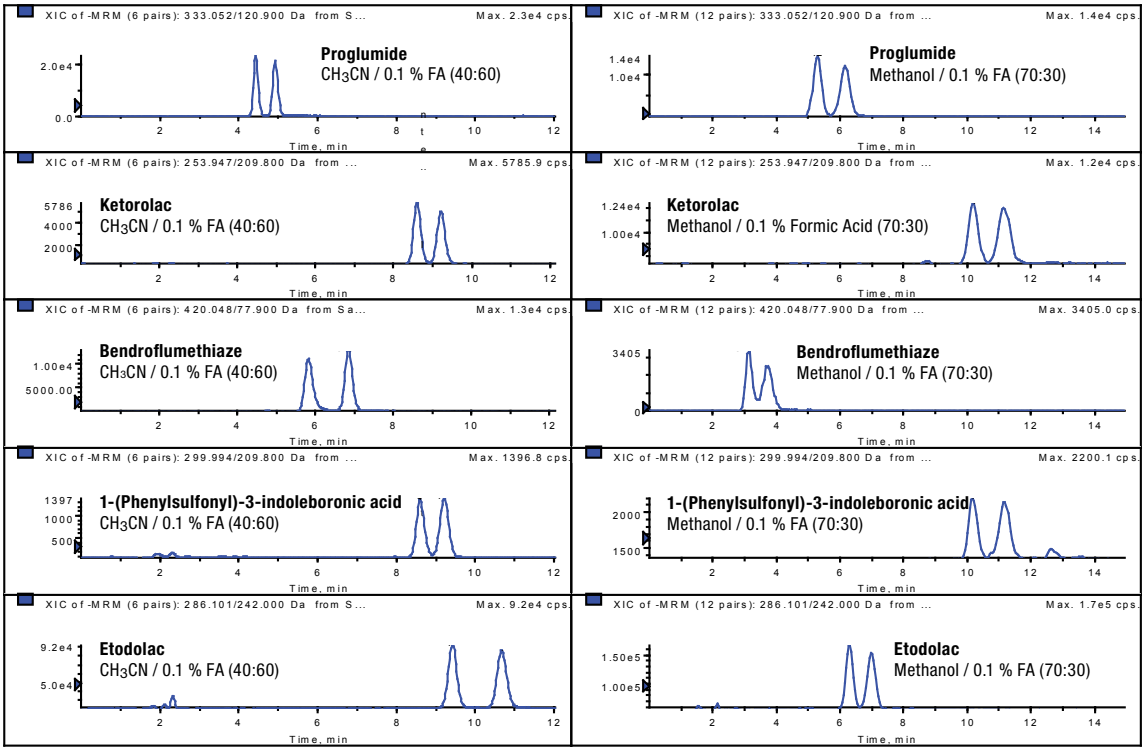
Proglumide
Lux® 3 µm Cellulose-1 150 x 2.0 mm

Ibuprofen
Lux® 5 µm Cellulose-3 250 x 4.6 mm



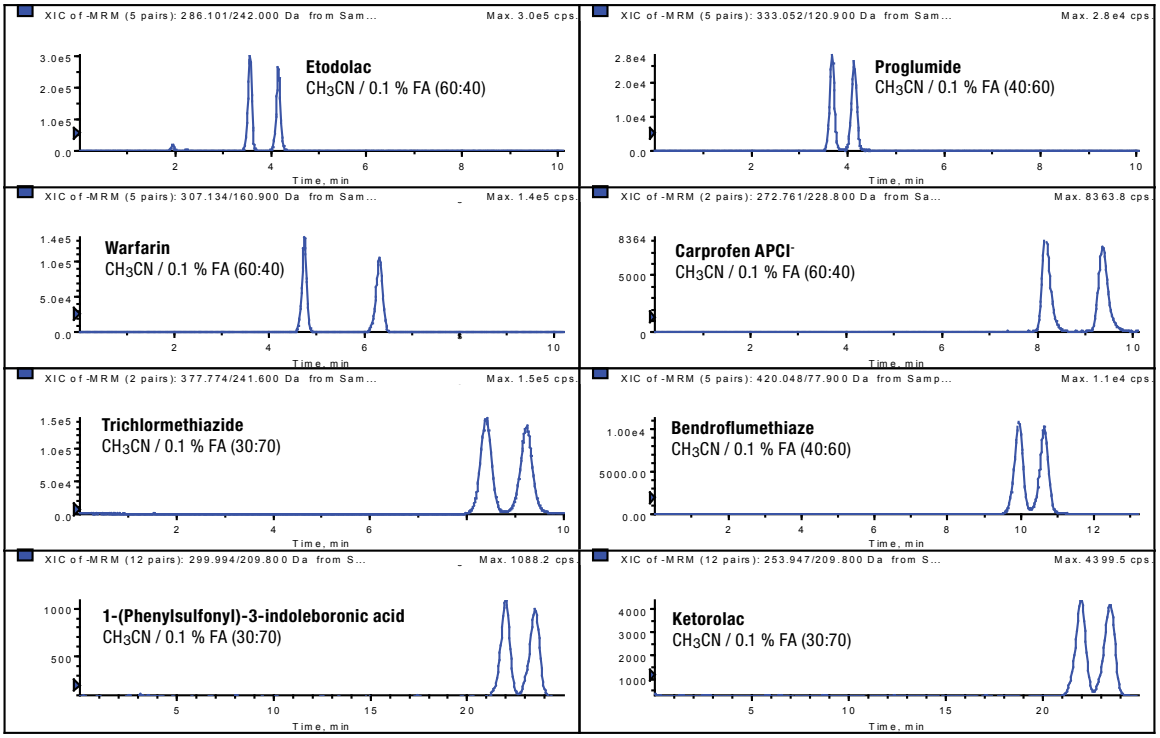
FA - Formic Acid

Figure 7. Enantioseparations on Lux Cellulose-2 with Acetonitrile or Methanol as Modifier



FA - Formic Acid

Figure 8. Enantioseparations in RP on Lux Cellulose-1 with Acetonitrile



FA - Formic Acid

Figure 9. Enantioseparations in RP on Lux Cellulose-4 with Acetonitrile

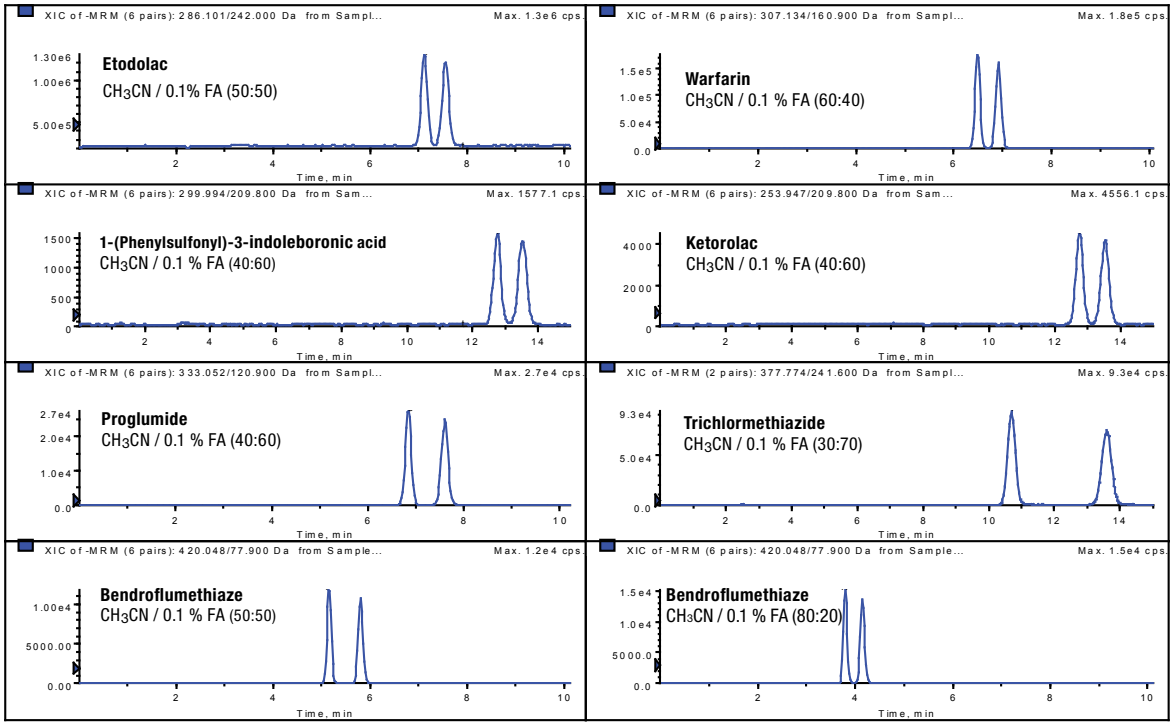


Figure 10. Enantioseparations in RP on Lux Amylose-2 with Acetonitrile

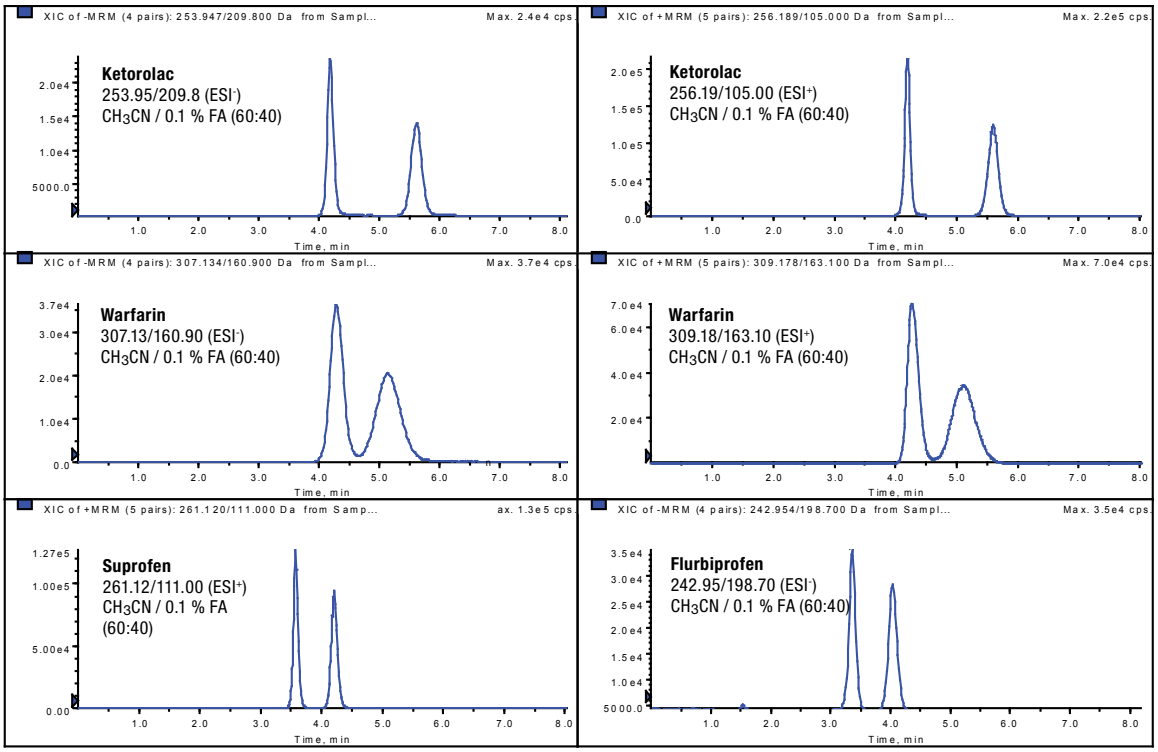
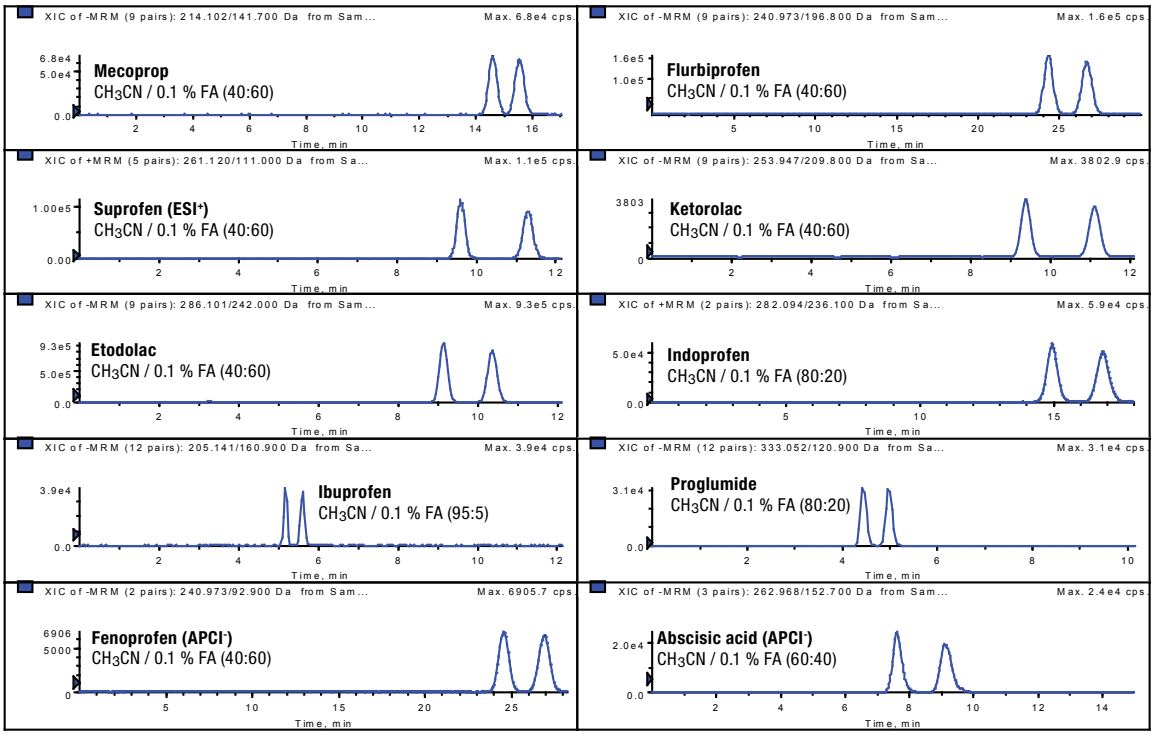


Figure 11. Enantioseparations in RP on Lux Cellulose-3 with Acetonitrile Modifier



Results and Discussion

Chiral LC/MS/MS experiments

Five different polysaccharide-based chiral stationary phases – Lux® Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 (**Figure 1**) were explored in the reversed phase elution mode for the separation of a variety of acidic compounds of pharmaceutical interest in mobile phases made of 0.1 % formic acid (FA) with acetonitrile or methanol as organic modifier and MS/MS detection.

Selection of mobile phase additives

As acidic analyte presents as anion ion at the neutral pH, which is not suitable for retaining on polysaccharide-based CSPs and resulting in early elution without enantiseparation, the acidic additive is often used as a counter ion in mobile phase for suppression of dissociation of acidic analyte to increase the elution time and enantioselectivity of acidic racemates on CSPs. Three acids - trifluoroacetic acid (TFA)- pK_a 0.59; formic acid (FA) - pK_a 3.75; acetic acid HAc) - pK_a 4.76 were evaluated on Lux Cellulose-1 and Lux Amylose-2 CSPs as acidic additives. In general, these additives provide similar enantioresolution for weakly acidic racemates (**Figure 5, a, b, c**) while the stronger acidic additive (TFA) performs better for the stronger acidic racemates (**Figure 5, d, e, f**). Formic acid provides comparable enantioseparations and peak shapes to TFA. Considering the "memory effect" of TFA on polysaccharide-based CSPs and its ion suppressing tendency in MS detection, formic acid is preferred over TFA in RP MPs for the chiral separation and MS/MS detection of acidic racemates.

Effect of mobile phase additives

The LC/MS/MS responses of acidic racemates with ESI in negative mode in 5 mM $HCOONH_4$, 5 mM CH_3COONH_4 and 5 mM NH_4HCO_3 containing mobile phases with either acetonitrile or methanol as organic modifier using an achiral column – Kinetex® C18 – were compared to responses in 0.1 % FA (**Figure 3 and 4**). The results show that MS/MS responses are comparable in 0.1 % FA CH_3CN or MeOH to the responses of analytes in other additives considerd here in the ESI- mode. This proves that 0.1 % FA as acidic additive in mobile phases is compatible for MS/MS detection and favored for enantioseparation of acidic racemates, except for carprofen which showed very poor responses in all of the additives at ESI^- mode. This proves that 0.1 % FA as acidic mobile phase additive is fully compatible with MS/MS detection of acidic racemates and can be implemented as the first choice.

Effect of organic modifier on chiral resolution

Acetonitrile or methanol was used in chiral RP HPLC separations as organic modifiers. Decreasing the eluting strength of the mobile phase by decreasing the portion of acetonitrile or methanol in the mobile phase will increase retention and resolution (**Figure 6**). However, once enantiomers elute later than 10 minutes with only partial resolution, baseline separation can rarely be achieved by further decreasing the % organic modifier in the mobile phase. In our study, acetonitrile was more successful in providing chiral resolution on Lux CSP in RP mode compared to methanol (**Figure 7**).

Chiral LC/MS/MS applications

Figures 8-11 demonstrate 15 chiral separations on Lux® CSPs. $APCI^-$ source was employed for the MS/MS detection of carprofen, abscisic acid, and fenoprofen as $APCI^-$ provides much better MS/MS signals than ESI^- for these three acidic racemates. ESI^+ mode was used for the MS/MS detection of suprofen. Most compounds evaluated here eluted in less than 10 min with baseline resolution in mobile phases of various eluting strength. The results show that Lux® Cellulose-3 was most successful in separating acidic racemates (10 racemates), especially non-steroidal anti-inflammatory racemates.

Conclusions

Fifteen successful chiral LC/MS/MS analyses are demonstrated for acidic racemic compounds on the polysaccharide-based CSPs Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 in reversed phase elution mode.

Formic acid as acidic additive in mobile phases is compatible for MS/MS detection and favored for enantioseparation of acidic racemates in RP.

Increasing the volume fraction of organic modifier (acetonitrile or methanol) in the RP mobile phase has the expected effect: it decreases retention and enantioselectivity; adjusting the organic modifier content of the mobile phase is essential to optimizing chiral resolution.

References

1. Liming P, Tivadar F, Swapna J, Chirality 2011 poster
2. H.R. Buser, M.D. Müller, Anal. Chem. 64 (1992) 3168
3. M.L. de la Puente, J. Chromatogr. A 1055 (2004) 55
4. S.M.R. Stanley, W.K. Wee, B.H. Lim, H.C. Foo, J. Chromatogr. B 848 (2007) 292